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Review Article

Technology Readiness Level of biodiversity monitoring with molecular methods – where are we on the road to routine implementation?

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Abstract

Human activities are causing rapid biodiversity loss across ecosystems, affecting human well-being and crucial ecosystem services. Traditional biodiversity monitoring tools cannot keep up with the increasing demands of monitoring due to their limited spatial or temporal coverage, high costs, and lack of taxonomic expertise. Thus, implementation of novel molecular monitoring methods such as environmental DNA (eDNA) and DNA metabarcoding, are necessary.

Molecular monitoring methods offer significant benefits for biodiversity monitoring and environmental assessment: high sensitivity and accuracy, non-invasive sampling, broad taxonomic range and cost and time efficiency. However, the diverse methodological approaches lead to poor comparability between studies and surveys, highlighting the need for standardised assessments.

We used the Technology Readiness Level (TRL) framework to evaluate the maturity of molecular monitoring methods, providing a structured assessment of their readiness for routine use. In a systematic literature review, 420 articles fulfilling the study criteria were assessed and both individual studies and method categories ranked according to the TRL scale. The findings revealed a growing number of studies, particularly in aquatic environments, with most studies validating molecular technologies on a small scale but lacking large-scale system demonstrations. Aquatic eDNA-based methods targeting fish showed overall higher technology readiness compared to other sample types and taxa and applications of molecular monitoring methods ranked into the highest TRL were predominantly freshwater studies.

Key barriers to the broader implementation of molecular methods to monitoring include the need for international standards, better quantitative estimates and comprehensive reference libraries. National and international cooperation is crucial for establishing common standards, ensuring reliable and comparable results and expediting the routine use of molecular methods in biodiversity monitoring. Recent efforts towards international standardisation are encouraging, but further coordinated actions are necessary for the global implementation and acceptance of these methods.

Key words: DNA metabarcoding, eDNA, molecular monitoring methods, standardisation, Technology Readiness Level

Introduction

Major and fast biodiversity losses occur across various ecosystems due to direct and indirect human impacts on nature. Many of these changes have negative and unpredictable reciprocal effects on both human well-being and crucial ecosystem services (IPBES 2018) which necessitates the development of methods to accurately measure their extent and provide reliable data about the effectiveness of measures taken in response to detrimental anthropogenic changes. Monitoring biodiversity is essential for the protection, conservation and restoration of ecosystems, especially given the current challenges such as climate change, habitat loss and globalisation (Baird and Hajibabaei 2012). The current traditional tools for monitoring ecosystem changes, such as sampling and taxonomic identification of target organismal groups, are insufficient. This is due to their poor spatial or temporal coverage, the lack of taxonomic expertise and intrinsic time and monetary costs involved. Thus, there is a need for new monitoring methods to fill these gaps and accurately extend our ecosystem level understanding on anthropogenically induced changes.

The importance of biodiversity monitoring and need for high-quality, accurate and timely data is becoming increasingly apparent across different sectors in society. A notable development is that, alongside traditional stakeholders such as authorities, natural resource managers and researchers, private sector actors from natural resource-dependent businesses are also increasingly calling for reliable biodiversity data to assess both their detrimental impacts on nature and to identify emerging positive effects of the use of sustainable practices (Kareiva and Marvier 2012). Novel monitoring methods, such as environmental DNA (eDNA) and other molecular monitoring methods, sometimes combined with high throughput analysis of in situ samples, have been identified as a very promising future technology (Hering et al. 2018). In addition to vastly improved community data, molecular monitoring methods can provide additional, previously unavailable data on monitoring genetic diversity and the use and development of indicators on genetic aspects of biodiversity (see Hoban et al. 2020).

The use of molecular methods for various environmental and biodiversity monitoring cases has grown exponentially during recent years. Concurrently, prices of lab analysis costs have declined from thousands of euros to a few cents per sample (Wetterstrand 2023). A downside of the rapid growth in diverse molecular monitoring approaches is that methodological differences amongst different methods can lead to weak overall comparability between studies, monitoring programmes and surveys. Diminished comparability due to methodological pluralism affects all process steps, from field sampling (design and protocols) to lab (pipelines and protocols), bioinformatics (analysis and protocols), data repositories and their use (metadata and FAIR Findable, Accessible, Interoperable and Reusable standards).

It is important to understand and assess advantages and disadvantages of different methods chosen for each process step in a systematic way to estimate their maturity (i.e. technology readiness) and to provide recommendations for

their applicability to existing traditional monitoring schemes. The Technology Readiness Level (TRL) is a commonly used approach to estimate maturity of any technology for routine use (European Association of Research & Technology Organisations 2014). In our specific context, TRL provides a logical template for an objective assessment of the maturity of various methodological steps for molecular monitoring schemes and helps to assess the degree of progress made towards implementation of molecular methods into routine use for biodiversity monitoring.

In this review, we aim to provide a comprehensive, literature-based situational overview of the use of molecular methods in biodiversity monitoring using a systematic approach. Specifically, our objectives are to: i) map eDNA and other molecular monitoring methods applications in recent biodiversity monitoring, ii) identify forerunners and potential best practices that are ready for transnational uptake and iii) outline pathways for standardisation of mature novel methods, which improve their comparability across various biodiversity monitoring activities.

We conducted an evaluation of the TRL (European Association of Research & Technology Organisations 2014) through a systematic review of the scientific literature published within the past seven years to: i) establish a comprehensive overview on how molecular methods have been recently used and ii) to evaluate their potential for further development and uptake into biological monitoring.

Material and methods

Systematic literature review

We conducted a search for publications published between 15 April 2017 – 6 November 2023, in the Web of Science database using the search string: "(TS = ((eDNA OR (environmental AND DNA)) AND monitoring AND biodiversity)) AND LANGUAGE:(English) AND DOCUMENT TYPES: (Article)" which resulted in 641 articles.

To facilitate team review of the research literature corpus, we used the systematic review protocol implemented in the CADIMA tool (https://www.cadima.info), which addresses the main issues commonly associated with literature reviews (Kohl et al. 2018). Articles were screened against predetermined study selection criteria and cross validation of reviewers was undertaken to harmonise results. Study selection was performed primarily based on the abstract, but augmented by referring to the full text where necessary. We set the following inclusion criteria:

i. The article is an original research paper.
ii. The study applies molecular methodology.
iii. The molecular methodology is used to assess the presence and/or abundance of one or several target taxa or to assess the status of the environment. By contrast, population genetic studies of individual target species were not included.
iv. The study discusses the topic of applying the adopted methodology in monitoring.
v. At least some of the analysed samples have been collected from an outdoor environment.

For each paper meeting the study selection criteria, we extracted a predetermined set of data fields based on the full texts that were used to evaluate the TRL that the paper represents. The extracted data included key parameters such as but not limited to: i) the methodology used, ii) the taxonomic, iii) spatial and iv) temporal scope of the study, v) whether the molecular methodology was compared to another methodology (e.g. morphology-based identification), vi) whether the molecular methodology was recommended by the authors for routine monitoring and vii) under what conditions (e.g. methodological challenges that still need addressing). An example of the extracted data sheet can be found in Suppl. material 2.

The Technology Readiness Level

Based on the extracted data, we assessed the TRL of the method used in each paper with respect to its implementation in routine monitoring. We used original TRL classes as described in European Association of Research & Technology Organisations (2014) (Fig. 1) as a cornerstone of our classification, but adopted more specific criteria for the assignment into each TRL class to assess the current technology readiness of molecular monitoring methods as reflected by the scientific literature.

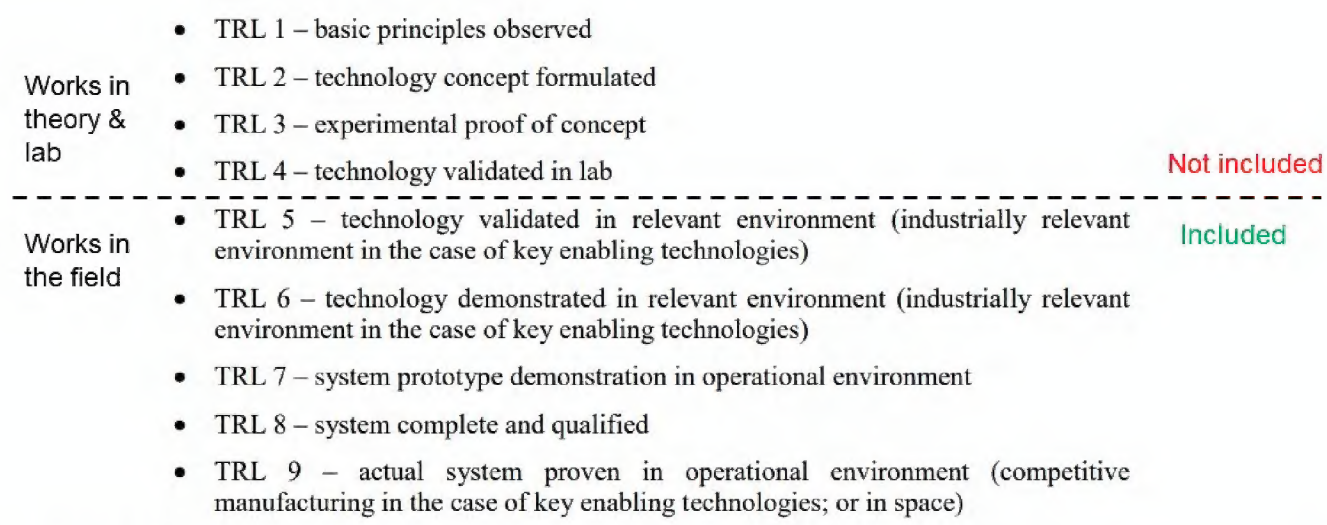
Works in theory & lab
TRL 1 – basic principles observed
TRL 2 – technology concept formulated
TRL 3 – experimental proof of concept
TRL 4 – technology validated in lab
Not included
Works in the field
TRL 5 – technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies)
TRL 6 – technology demonstrated in relevant environment (industrially relevant environment in the case of key enabling technologies)
TRL 7 – system prototype demonstration in operational environment
TRL 8 – system complete and qualified
TRL 9 – actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies; or in space)
Included

Figure 1. Original TRL classes as described in European Association of Research & Technology Organisations (2014). TRL class 5 was the cut-off for study selection and only TRL classes 5–9 were included in our study.

TRL class 5 was the cut-off for study selection. For the review, TRLs included were interpreted using the following progressively applied criteria:

TRL5 (Technology validated in relevant environment)

***Criterion*:** This was the minimum level reached by all studies that met our study selection criteria. ***Interpretation*:** The methods used in these studies are relevant for monitoring and appear technically feasible for routine use under relevant outdoor conditions.

TRL6 (Technology demonstrated in a relevant environment)

***Criterion*:** The molecular monitoring method is compared to another established ("traditional") method and is considered to produce either equal results or to have advantages (e.g. cost-efficiency, improved detection probability

of species or more comprehensive monitoring of the species community). ***Interpretation*:** The method has been shown to produce meaningful results in the relevant environment.

TRL7 (System prototype demonstrated in operational environment)

***Criteria*:** The molecular method is applied at a medium or large spatial scale (> 10 km maximum distance between sampling sites), is based on at least 20 samples and its implementation in monitoring is at least conditionally recommended. ***Interpretation*:** The jump in the TRL scale from a "technology" to a "system" has been interpreted in terms of scale, i.e. a technology can be demonstrated by sampling at individual locations, but to meet the criteria of a system, the method should be scalable. To qualify for this level requires that the study demonstrates scalability.

TRL8 (System complete and qualified)

***Criterion*:** The molecular method is directly compared to the prevailing traditional method (i.e. with comparable samples) and its implementation is recommended without limitation. ***Interpretation*:** To qualify, the results of the monitoring system should be compared directly to an existing method. Consideration was given that for some taxa readily identified by molecular methods, a comparison to traditional methods is not feasible. Thus, a recommendation of implementation without major limitations, for example, on environmental conditions, was interpreted to reflect the required technology readiness.

TRL9 (Actual system proven in the operational environment)

***Criterion*:** The paper states that the molecular method is already implemented in an existing legislative monitoring program. ***Interpretation*:** The molecular method is used in actual operational monitoring, proving the feasibility of the method at scale also including solutions for representative sampling design, data recording and organisation of sampling.

Data analysis

Paper-based analysis

Using the above criteria, each of the reviewed papers was assigned a TRL value. We then visualised the distribution of TRL values in relation to different ecosystems (freshwater, marine and terrestrial), application categories (e.g. biodiversity, threatened species or harmful species monitoring) and organism groups, as well as the change in TRL over time. For illustrative and statistical purposes, we manually classified the specific descriptions of the application and target group recorded for each paper into broader categories for both the "application" and "organism group" categories. To assess the statistical significance of the factors explaining paper-specific TRL, we fitted the linear model "TRL ~ publication year + ecosystem + application category + organism group" to the paper-specific data (n = 420) assuming normally distributed errors using the LinearModelFit

function of the Wolfram Mathematica software (Wolfram Research, Inc.). Publication year was modelled as a continuous variable, while ecosystem, application and organism group were categorical variables with 3, 6 and 6 different levels, respectively. In addition, we fitted three alternative models each including an additional interaction term ("year*ecosystem", "year*application category" or "year*organism group"). However, as the interaction term was never significant according to an analysis of variance and the performance of the models with interaction terms was lower than that of the additive model as measured by the AIC and BIC criteria, we report only the results of the additive model.

Methodology-based TRL

In addition to assessing the TRL of each individual paper, we also classified papers representing different methodologies and determined the TRL reached by each methodology as the maximum paper specific TRL value within the class. Here, a methodology was defined as a unique combination of: (i) the broad molecular methodology (DNA metabarcoding or a PCR-based approach such as qPCR or ddPCR), (ii) sample type (e.g. water, soil, sediment) and (iii) organism group. Molecular methods other than DNA metabarcoding and PCR-based methods (e.g. DNA metagenomics or RNA-based methods) were represented only by a small number of papers and were not included in the methodology-based TRL assessment.

Results

A total of 641 research papers published between 15.4.2017 and 6.11.2023 were screened against predetermined study selection criteria in the CADIMA tool, resulting in 420 research papers fulfilling the criteria. The number of papers published increased annually during the study period. In 2018, the first full year of our search period, 42 papers which met our search criteria, were published. The number of published papers per year has almost doubled within our search window with 80 published papers fulfilling the criteria in 2022, the last full year of our search period (Fig. 2). The number of studies performed in the freshwater environment varied from 17 in 2019 to 34 per year in 2022. In marine environments, the minimum number of papers was 12, all of which were published in 2018, whereas the maximum, i.e. 34 papers were published in 2023. Comparably, in terrestrial environments, only six papers were published in 2018 and the maximum number of published papers for terrestrial environments was 18 in 2021. Note that the amount of published terrestrial papers per year decreased to 14 and 15 published in 2022 and 2023, respectively.

Most of the study sites were located in the United States (54 studies), followed by China (43), Australia (31) Japan (30), France (21), Canada (20), Germany (17), Denmark (17), New Zealand (15) and The United Kingdom (14) (Fig. 3). Only 30 studies spanned sites over multiple countries, whereas most studies were conducted only in one country.

The internationally published scientific research on molecular monitoring methods within the last seven years is heavily dominated by application cases to aquatic environments (Fig. 4). There is an equal proportion of freshwater to marine studies reporting applications that rank at TRL 6 and 7. Fewer studies

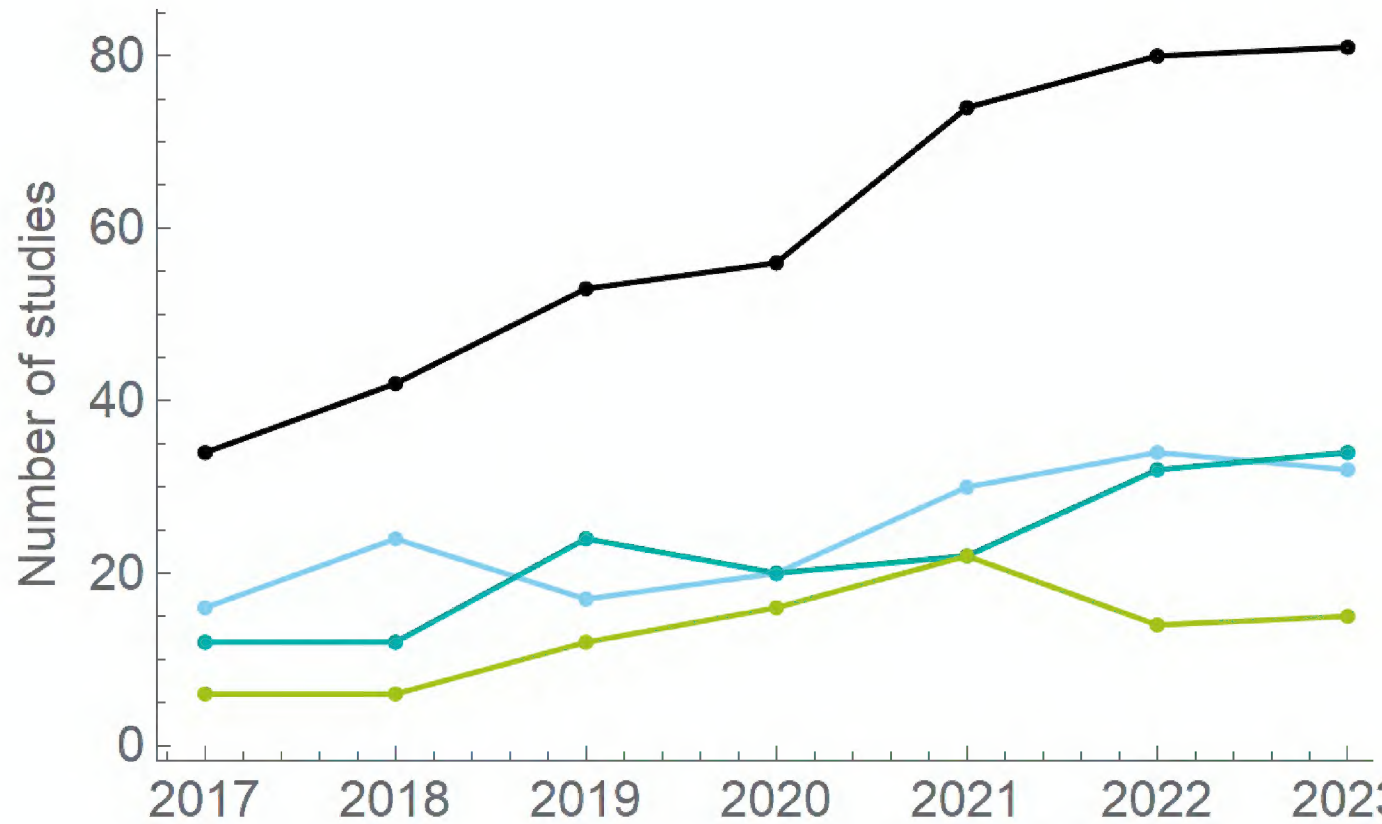

Figure 2. Number of original research papers published per year during the search period 15.4.2017–6.11.2023 in different study environments (black = all, light blue = freshwater, turquoise = marine, green = terrestrial).

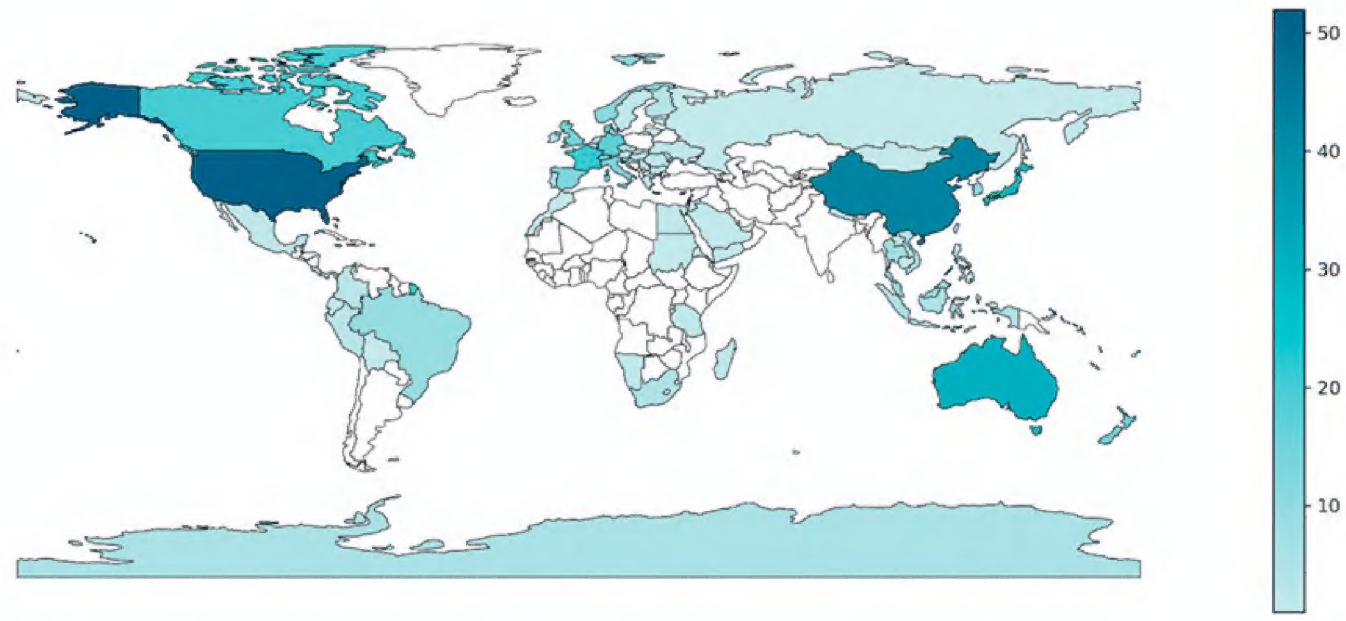

Figure 3. Geographical distribution and incidents of the research papers.

scored higher than TRL 7 and those that did were strongly dominated by freshwater studies. Aquatic environments also provided the only two examples of the highest Technology Readiness Level (TRL 9, already implemented), which included invasive fish species (Carim et al. 2020) and benthic invertebrates (Aylagas et al. 2018). Another example of a high TRL is the routine eDNA-based monitoring of the great crested newt (*Triturus cristatus*) in Great Britain (Biggs et al. 2015).

Overall, fish and invertebrates were the two most actively studied groups. The dominant pattern in the data is that TRL classes 5–9 seem to follow a normal distribution, with most of the studies falling into categories 6 and 7, indicating that, while the methods are now broadly validated in small-scale field studies, systematic large-scale demonstrations are still scarce. This pattern was very robust across different ecosystems, application categories and organism groups and, interestingly, also did not markedly evolve over the seven years included in our study (Fig. 6). The results of the statistical analysis confirm that year, ecosystem and application category did not significantly explain variation in TRL (Table 1) and that, overall, the explanatory power of the statistical model was very low (R^2 = 0.06).

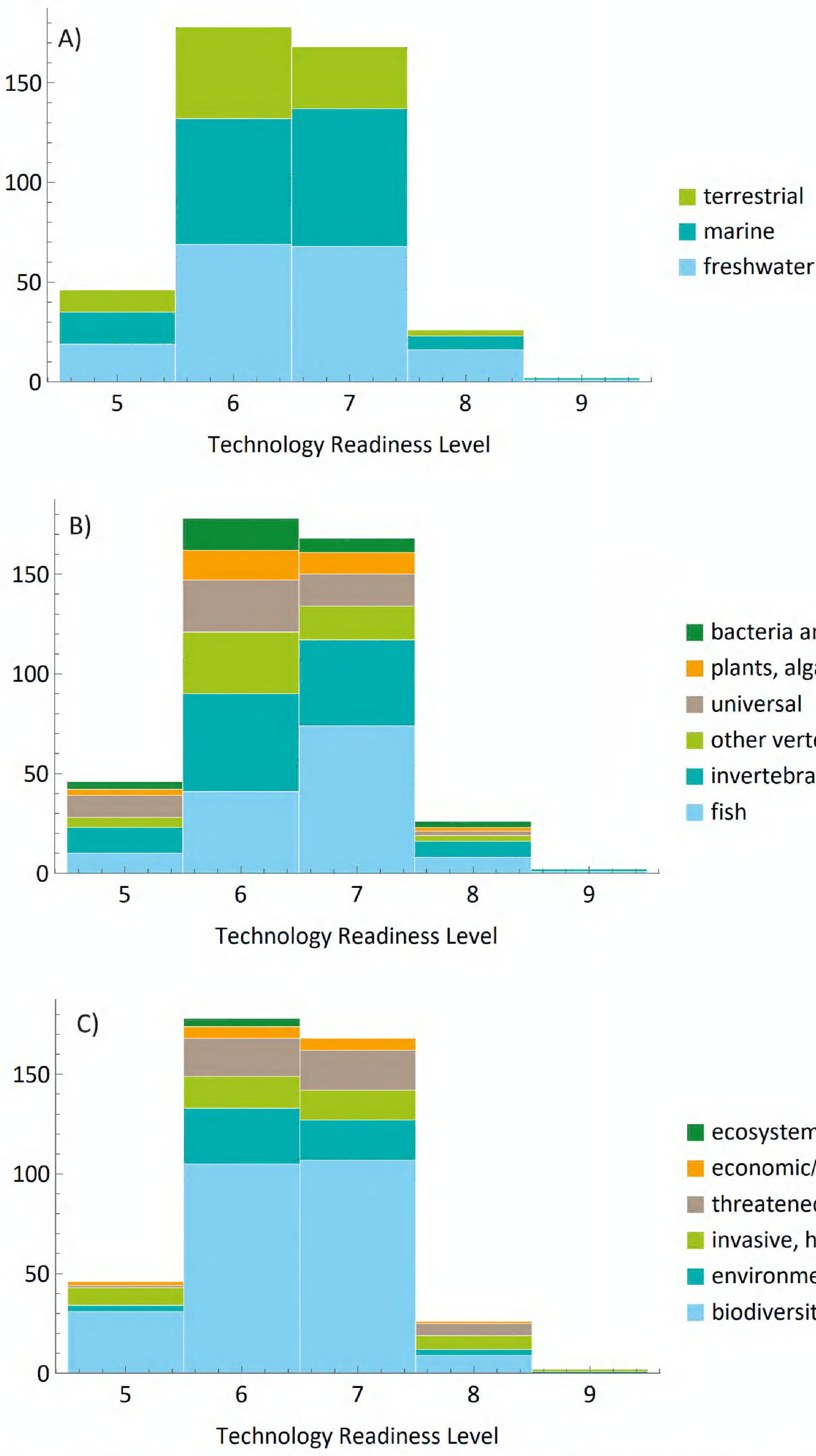

Figure 4. Distribution of the 420 original research papers published between 2017 and 2023 according to Technology Readiness Levels (TRL) of molecular monitoring methods classified by **A** ecosystem **B** organism group and **C** primary monitoring application represented by the study.

Table 1. Results of the statistical analysis examining the role of different factors in explaining the Technology Readiness Level represented by individual studies (n = 420). Note that the levels of the categorical variables (ecosystem, application category and organism group) are the same as those illustrated in Fig. 4.

Model	**TRL ~ year (continuous) + system (categorical; 3 levels) + application (categorical; 6 levels) + group (categorical; 6 levels)**				
R^2	0.05947				
ANOVA table					
	DF	SS	MS	F-Statistic	P-Value
year	1	0.555	0.555	0.93	0.337
system	2	2.517	1.258	2.1	0.124
application	5	4.557	0.911	1.52	0.182
group	5	7.765	1.553	2.59	0.025
Error	406	243.463	0.6		
Total	419	258.857			
Parameter estimates					
	Estimate	Standard Error	t-Statistic	P-Value	
1	-27.64	42.54	-0.65	0.516	
year	0.02	0.02	0.8	0.425	
system ["freshwater"]	0.04	0.11	0.4	0.688	
system ["marine"]	0.07	0.11	0.62	0.533	
application ["biodiversity"]	-0.27	0.13	-2.09	0.037	
application ["economic/key species"]	-0.37	0.23	-1.61	0.109	
application ["ecosystem structure and functioning"]	-0.66	0.41	-1.61	0.108	
application ["environmental status assessment"]	-0.08	0.17	-0.49	0.628	
application ["invasive, harmful and pathogen species"]	-0.14	0.17	-0.85	0.399	
group ["bacteria and fungi"]	0.08	0.18	0.46	0.646	
group ["fish"]	0.44	0.13	3.33	0.001	
group ["invertebrates"]	0.26	0.13	1.98	0.048	
group ["other vertebrates"]	0.15	0.16	0.98	0.325	
group ["plants, algae, phytoplankton"]	0.22	0.18	1.23	0.218	

According to the analysis of variance, the organism group was the only significant factor and the estimated model parameters show that this effect can be mostly attributed to the higher TRL in studies targeting fish (Table 1, Fig. 4).

Metabarcoding-based approaches were used in 338 studies and PCR-based methods, such as quantitative PCR (qPCR) or Droplet Digital PCR (ddPCR) 80 studies. Several research papers used both approaches. In Fig. 5, all research papers that used metabarcoding approaches were classified into "metabarcoding" regardless of the possible use of additional PCR-based approaches.

The distribution of TRL classes across different environments and years (Fig. 6) shows only minor changes in the inter-annual distribution of TRL for original papers across years. The most significant change in TRL class is observed in marine environments.

In the reviewed papers, lack of standardisation was often mentioned as one of the key restricting factors for the larger implementation of molecular monitoring methods (see, for example, Agersnap et al. (2017); Baldigo et al. (2017); Gargan et al. (2017); Vasselon et al. (2017); Minerovic et al. (2020); Suter et al. (2021)). Need for further method optimisation, improved quantitative estimates and development of reference libraries are also often mentioned as restricting factors for the larger implementation of molecular monitoring methods (see, for example, Vasselon et al. (2017); Schnell et al. (2018); White et al. (2020)).

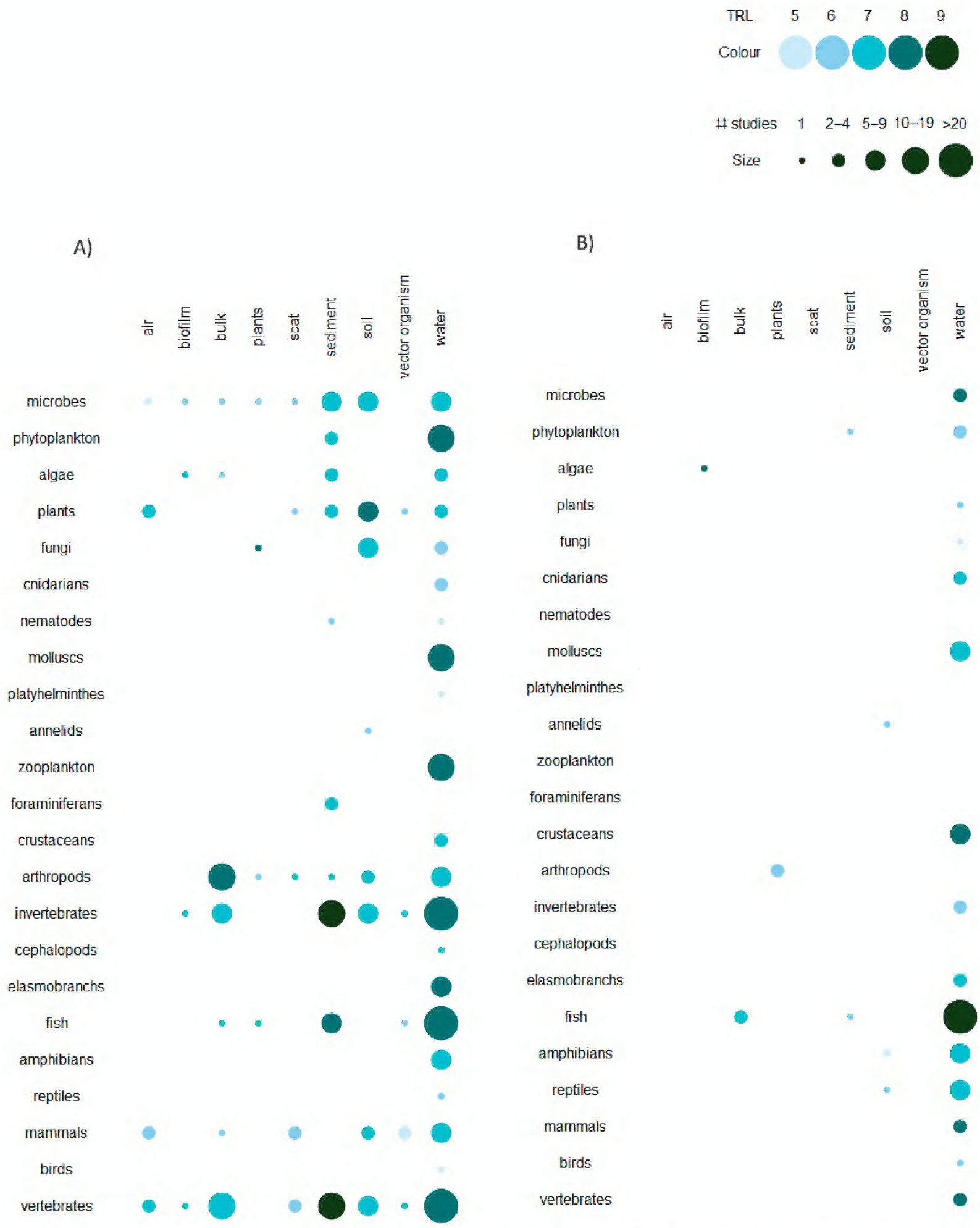

Figure 5. Technology Readiness Level for different combinations of organism group and sample type for **A** metabarcoding and **B** PCR-based methods. Circle size represents the number of studies within each category and circle colour the maximum Technology Readiness Level reached amongst those studies.

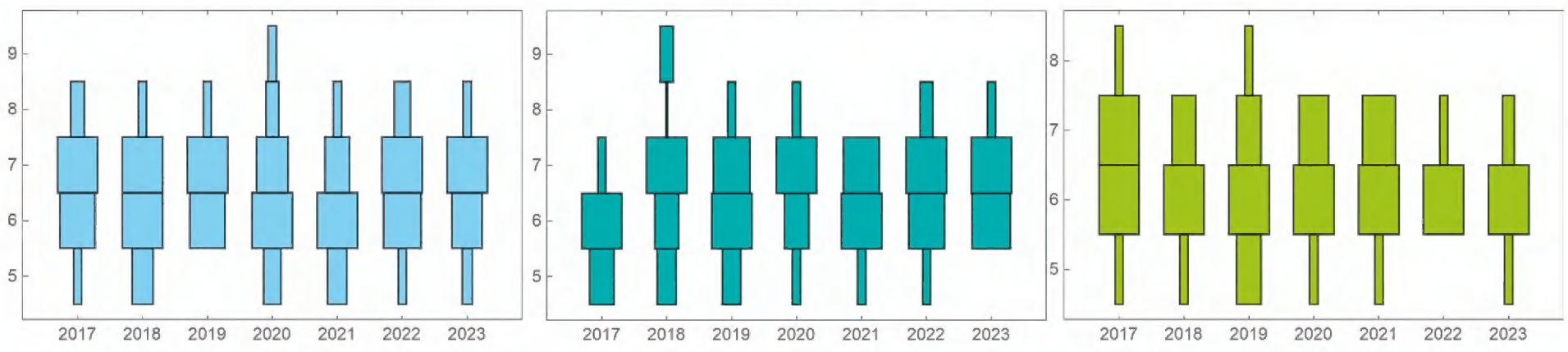

Figure 6. Distribution of TRL classes across years and freshwater (light blue), marine (turquoise) and terrestrial (green) environments.

Discussion

The increased demand created by environmental legislation and international treaties for more accurate and timely information on the state of the ecosystem overburdens current traditional monitoring methodologies and has created the need to look for novel monitoring and analytic solutions. Molecular identification techniques have great potential to improve and extend current biological monitoring in all types of habitats. Unmonitored changes in patterns of biodiversity in response to global megatrends (e.g. climate change, urbanisation, invasive alien species, increasing chemical stress on soils and groundwaters) stand to benefit from the speedy uptake of these methods into routine monitoring (Thomsen and Willerslev 2015).

Further, their application, combined with traditional monitoring and assessments, could improve the accuracy of monitoring results and ensure that appropriate management actions are taken and potentially increase spatial coverage. Molecular monitoring methods can produce objective, easily comparable and reproducible species identification and can be used in large-scale monitoring (Bohmann et al. 2014). Molecular monitoring methods can reliably detect and be used to monitor currently hard-to-detect and poorly-known groups of organisms (e.g. aquatic and soil microbes, fungi, certain groups of insects) that are currently excluded from monitoring based on the shortcomings of traditional taxonomic identification methods (e.g. Abrego et al. (2018); Frøslev et al. (2019)). Some molecular monitoring methods already represent the better choice, compared to traditional methods, for reliable mapping of intraspecific genetic diversity, the conservation of which is increasingly acknowledged internationally (Secretariat of the Convention on Biological Diversity 2020; Hoban et al. 2020).

To produce meaningful results and to attain high TRL, molecular monitoring methods need reliable reference databases and reliable, specific genetic tools for a broad range of organisms and commonly agreed upon minimum criteria for methodological and analytical pipelines. Several commonly used methods (e.g. metabarcoding) have evolved from the prototype stage (TRL 3–4) to TRL level 6–8 where the technical operation has been demonstrated in relevant settings (e.g. Meissner et al. 2019).

Despite their demonstrated success and benefits, applications of molecular identification methods have mainly been limited to proof of concept or validation projects as is reflected by our data which ranked the bulk of studies using molecular monitoring methods below TRL 8. Many authors state that the transition from TRL level 7 to TRL 8 or TRL 9, i.e. method uptake into routine use, is rarely limited by actual technical problems in upscaling of molecular monitoring methods to larger scales. Rather, routine legislative uptake of molecular methods is often stated to be restricted by roadblocks related to “legitimisation” and “legalisation” i.e. acceptance and regulatory readiness levels (sensu Vik et al. 2021), such as the lack of method standardisation.

Innovation processes are complex, evolutionary, relational, temporal and cultural. Trust development is dynamic across individual and organisational levels (Garud et al. 2013; Schilke and Cook 2013). For molecular monitoring methods, managing these complexities and building trust amongst stakeholders and end-users are crucial as the technology evolves from lab validation to operational environments. Understanding how innovations are adopted by different

groups helps tailor communication and engagement strategies (Rogers 1962; Doz 1996). When implementing molecular monitoring methods, the phases of negotiation, commitment and execution, mediated by ongoing evaluation, are crucial. To implement new monitoring methods into routine use, stakeholders need to negotiate and agree on standardised protocols, commit resources and iteratively test and, consequently, refine the technology. Continuous process and method evaluation ensures progress and gradual method adaptation. Strategic alliances or organisations and platforms that facilitate cooperation in strategic alliances often evolve through commonly identified initial conditions and joint or co-learning processes (e.g. Doz 1996).

To increase acceptance and regulatory readiness levels further, there is an urgent need for both national and international cooperation including cross collaboration with technology and knowledge transfer experts, social scientists and economists to expedite the routine implementation of molecular methods for legislative monitoring. Ensuring the development of molecular monitoring methods is cost-effective and fair is crucial. This includes making the technology accessible to various regions and distributing the benefits of advanced monitoring equitably (Ring and Van de Ven 1994). More generally, three modes of action with respect to the implementation of molecular monitoring methods into routine legislative use exist: i) focus on national or regional cooperation to produce guidelines, ii) the “wait and see” option and iii) strengthening of international cooperation to develop common standards. We will briefly discuss each one in the following.

Strengthening regional or national cooperation has several short-term advantages, but also entails historically proven drawbacks. National guidelines often are much easier to develop than international ones since the number of stakeholders that need to be engaged is often more limited. Thus, time spent on efforts to develop and reach consensus on national or regional guidelines and to implement methods may be reduced. It is important to recognise that this initial timesaving aspect only prevails if the endpoint of the analysis is indeed only national or regional. However, for biodiversity monitoring or in bioassessments, for example, of the status of waterbodies, national assessments are just one goal. In Europe and globally, methods and data collected by them often need to answer more than single national scale questions on patterns of biodiversity or the state of the environment. Producing accurate and comparable data to such multifaceted questions requires international cooperation.

Cooperation on international method or method standard development is often slower to begin with as the identification of stakeholders and the definition of an efficient engagement process of the relevant stakeholders takes more time. However, choosing a national approach to attain short-term time savings over an international one has multiple significant consequences when the ultimate goal is a global-level endpoint. The ability to directly compare results from one nation to another and to meaningfully combine them is decreasing with method complexity as independently developed national guidelines will have facets that will differ and create different end results. To be able to make international inferences on general patterns that several different national methods describe often requires intercalibration of results from these national methods. This is a far from trivial task, as the implementation of existing national methods to assess water quality in the EU aptly demonstrated. In the intercalibration

of methods for the Water Framework Directive (EC 2000), a total of over 300 national methods existed that required intercalibration which took over 20 years to complete and still resulted in often less than optimal comparability (Birk et al. 2012).

For molecular monitoring methods, a future of similarly laborious intercalibration can and should be effectively averted. The solution to both attaining higher TRLs and, thus, routine use involves international cooperation on defining minimum criteria for high TRL level application intended for use in routine biodiversity monitoring and bioassessment.

Currently, there is little coordination between national research organisations and other end-users, both for molecular monitoring methods specifically and for new environmental monitoring methods in general. Some prominent examples of national roadmaps for the implementation of molecular monitoring methods exist (e.g. Norros et al. 2022; De Brauwer et al. 2023; Goodwin et al. 2024; Kelly et al. 2024), which could be extended to other countries and regions. If these general roadmaps were combined with strategic implementation plans for specific methods that accounted for and integrated the pros and cons of molecular monitoring methods across various taxa, these novel methods could support and expedite transnational biodiversity monitoring schemes.

While national coordination around molecular monitoring methods is building up rapidly in many countries, the number of national key stakeholders is currently still relatively low. The fact that molecular monitoring methods intended for routine use provide data that are not only necessarily directed towards national endpoints creates a window of opportunity to choose international standardisation of minimum requirements for molecular monitoring method use as a common starting point.

By contrast, focusing development on national guidelines without concurrent international coordination of efforts entails a high risk of duplication of work, creating internationally incompatible solutions in a quickly evolving field and sidelining inputs of stakeholders from less advanced regions like the Global South, which are the regions holding most of the threatened biodiversity we globally seek to protect.

Several central European nations i.e. Germany, France and, in particular, Finland have taken an active role to advance the international standardisation of forerunner molecular monitoring methods for routine biological monitoring. In the past few years, these efforts have spawned European work to standardise sampling of eDNA from water and progressed to the creation of a dedicated working group in 2018, as well as the development of its first CEN standard (EN 17805:2023). The European decision to focus further standardisation efforts on periphytic diatoms and aquatic macroinvertebrate metabarcoding is strongly mirrored by our data.

After the ratification of the Kunming-Montreal protocol, the need for an international platform to advance the implementation of the global biodiversity Framework (GBF) was realised and met by establishing a dedicated Technical Committee (TC) for Biodiversity under ISO (i.e. TC 331) to develop international standards. However, this TC's scope did not specifically advance the minimum method requirements of molecular monitoring methods needed for routine implementation in biodiversity monitoring. Mirroring both our study results that the highest TRL of molecular monitoring methods are found in aquatic

environments and the desire to create international comparability to counteract scepticism about the reliability and reproducibility of environmental genomics metrics led to the establishment of a new working group under ISO TC 147 "Water quality" in 2023. The new working group is specifically dedicated to the international standardisation of minimum requirements of molecular monitoring methods for use in routine bioassessment.

To further facilitate inclusive access to method standard development for Global South stakeholders and to expedite the formulation of seed documents for introduction into ISO standardisation, the International eDNA Standardisation Task Force iESTF (https://iestf.global) was established in 2023. iESTF offers an inclusive platform that cooperates closely with the international research community and various other key stakeholders and transparently works on the creation of seed documents for specific steps of the molecular monitoring methods process.

These recent developments partly reflect the growing level of international interest and commitment of countries to the future routine and comparable implementation of molecular monitoring methods. However, both our data and current developments in the field of standard creation for marine and terrestrial environments lag behind. A clear danger is that similar developments in these environments will turn to national guideline creation at the expense of international standardisation which will delay the creation of internationally comparable biodiversity data for terrestrial and marine environments. Further coordinated work within and between different environments is needed to ensure unified application and interpretation of molecular monitoring methods in future global and national legislative monitoring.

Molecular monitoring methods have advanced to a stage where methods to target new target taxonomic groups are continually emerging. These new developments often begin with lower TRL-level activities. This could partly explain the observed distribution of TRL classes across different environments (Fig. 6).

Conclusion

Molecular monitoring methods have reached critical maturity and their implementation has started worldwide. Molecular monitoring methods have great potential to benefit, improve and extend current biological monitoring in all types of habitats. However, the field is fragmented causing a risk of unnecessary duplication of efforts, method pluralism and resulting incompatibility of the end results.

Based on the 420 papers assessed in this review, recent international research on molecular monitoring methods has predominantly focused on aquatic environments, with equal emphasis on freshwater and marine studies at Technology Readiness Levels (TRL) 6 and 7. High TRLs are mainly seen in freshwater studies, including the highest TRL 9 applications for invasive fish species and benthic invertebrates. TRL classes 5–9 roughly followed a normal distribution, with most of the studies falling into categories 6 and 7, indicating that, while the methods are broadly validated in small-scale field studies, systematic large-scale demonstrations and routine implementation are still scarce.

Restricting factors to the uptake of molecular methods into routine monitoring described in the reviewed papers were lack of standardisation, methodological

optimisation and comprehensive reference libraries. National and international cooperation is crucial to establish common standards and ensure consistent, reliable and comparable results. Recent international efforts and the establishment of international working groups indicate progress, but further coordinated action is necessary to achieve unified application and interpretation of molecular monitoring methods for biodiversity and bioassessment globally.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: PV, TL, VN, KM. Data curation: TL, VN, KMV, LN, PK, IP, KM, KK, SL, JM, JJ, PV, MT. Formal analysis: VN. Investigation: TL. Visualization: JM. Writing - original draft: TL, KM, VN, PV. Writing - review and editing: KK, IP, KMV, JM, JJ, SL, PK, LN.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Extracted CADIMA data

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Data type: xlsx

Explanation note: Extracted CADIMA data and TRL classes for 420 research papers published between 15.4.2017 and 6.11.2023.

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Link: https://doi.org/10.3897/mbmg.9.130834.suppl1

Supplementary material 2

Example of a filled data sheet on CADIMA - tool

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Data type: png

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